Performance Evaluation of Highly Multiplexed Microbiology/MCM Devices – End User Perspective

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Advancing Regulatory Science for Highly Multiplexed Microbiology/Medical Countermeasure Devices

FDA, MD   October 13, 2011

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NQF
National Quality Healthcare Award
Winner 2010

Laboratory and Clinical Acceptance: Necessity and Use

Unmet clinical need and/or service improvement

- Is it relevant to our patient population?
- Does it contain the appropriate scope of analytes?
- Will the test change clinical practice?
- Do clinicians want the test?
- Will they accept and use the test?
- How do we ensure appropriate use?
- How do we monitor usage?
- How do we educate our medical and nursing staff?
- Does the clinical benefit outweigh the cost?

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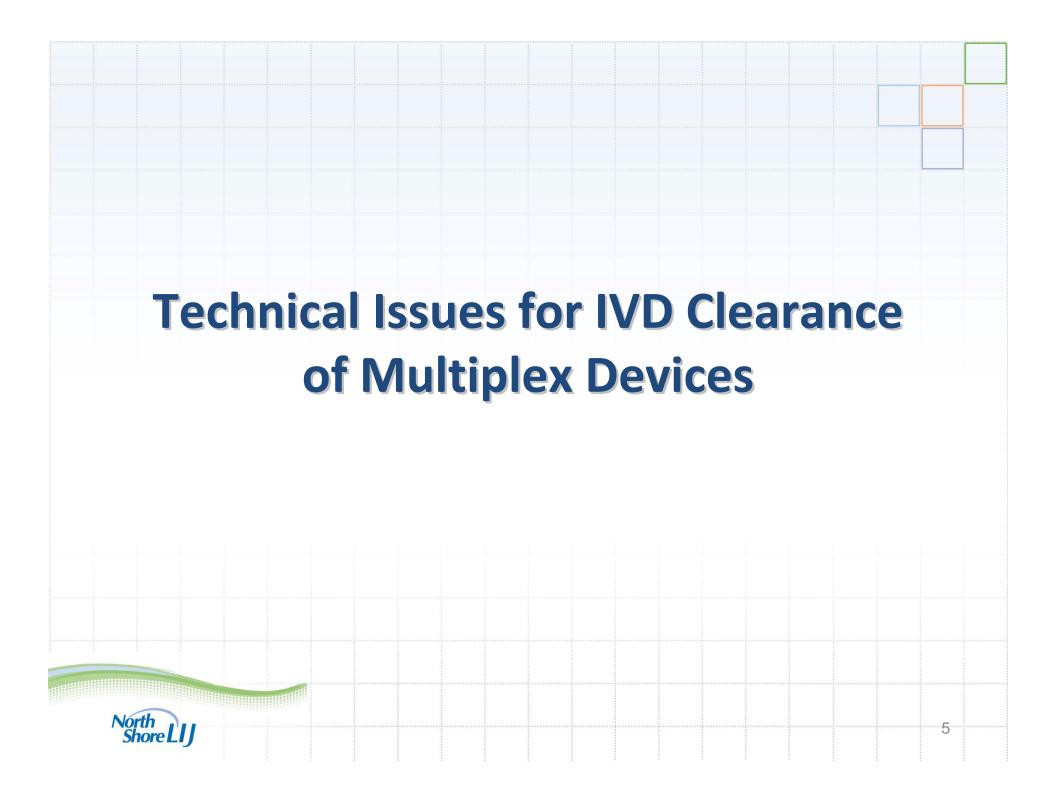
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Laboratory Acceptance

- **FDA Status**
  - Impact on regulatory requirements: verification/validation/QC vs LDT
  - CLIA, CAP, State
  - Expense, time, expertise to verify or validate
- **Performance characteristics**
  - Improves diagnostic yield (current and new analytes)
  - Comparable or better to current methods
  - Sensitivity, specificity, PPV, NPV
- **Expertise required to perform assay**
- **Desire for new and innovative technology**

Laboratory Adoption

- **Cost benefit ratio:**
  - Bring revenue to lab (out reach)
  - Save money
    - Replace a costly send out test
    - Save technical time
    - Combines multiple tests in one assay
    - Decrease in reagent costs
  - Increase in laboratory testing costs
    - Provides strong clinical benefit and "hospital" savings
- **Evaluate laboratory costs**
  - Instrumentation, technical time, reagents
- **Work flow/turn around time**
  - STAT vs batch, once a day, multiple runs, 24/7??
- **Space**

Technical Issues for IVD Clearance of Multiplex Devices

Composition of Multiplex Assays

- Comprehensive so supplemental testing is not required: replace not add on ($$$$)
- Must not be incrementally more expensive by analyte number
- Analytes must be clinically relevant for diagnosis, syndrome and/or patient population
- Option to limit test results: Software function
- Multiplex convenience should not result in decreased sensitivity of detection

Selection of Sample Type(s)

- **How many specimen types will require validation?**
- **Will the sample types be related (NP wash, NP aspirate, NP swab) or potentially highly diverse (CSF, urine, blood)?**
- **Among related types how many need individual validation and how many positives per type?**
- **Will sample type effect target stability prior to testing?**
- **Will certain sample types require pretreatment steps?**

Nucleic Acid Extraction

- **May require more stringent conditions for nucleic acid isolation and sample purity**
- **May need to recover a mixture of nucleic acids**
  - Ex: RNA and DNA viruses
- **Recovery at potentially variable clinically relevant levels:**
  - Colonization vs infection
  - Amount of target present during infection
  - Time of sample collection
- **Efficiency across all targets and sample types**
  - Removal of amplification inhibitors
  - Effect of interfering substances
  - Possibility of multiple targets (high and low titer)
- **Will input and extractions volumes vary by specimen type?**

Multiplex Considerations

- Complex assay parameters
- Test the multiplex system in its final format to assess:
  - target competition
  - cross reactivity among the different primers and probes
  - potential cross over of signals between analytes
- Validate each analyte per sample type
- Demonstrate equal detection of all potential targets, alone and in combination with other analytes detected simultaneously by the system
- How many potential positives/sample?

Need for an Internal Control?

- **May not be necessary if extraction removes >99% of inhibitors for EACH sample type to be tested**
  - Test numbers (n=?) of individual sample types with spiked target(s) at the LOD
- **Design an internal control that goes through the entire process (also serves as an extraction control)**
  - Low copy housekeeping gene (specimen quality)
  - Spiked IC (IVTs) at low copy ($\leq$10 fold over LOD)
  - Validates sample results
  - Non-competitive (impair sensitivity)
- **Establish inhibition rates per sample type using multiple individual samples (not pooled)**
- **Establish acceptable range for IC (not just positive)**

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Development of External Controls

- **Need to verify all reagents for each target**
- **Need to include controls in every run**
  - Need to verify all targets every run?
  - Test more controls than patients
  - Are process controls acceptable for single unit devices?
- **Should go through the entire test procedure**
- **Should mimic real samples as best as possible (be present in appropriate matrix)**
- **Should be tested at an analytically and clinically relevant level**

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External Controls

- **Difficult to find for rare analytes, laboratories unable to prepare own controls**
- **Come in the test kit**
  - Can not be used to validate that specific lot or shipment
- **Be provided external to kit by manufacturer**
- **Commercially available**
- **Should be part of the test development**

Availability of Validation Materials

- **Problems:**
  - **Rare targets**
  - **Seasonal targets**
  - **Organisms unable to grow in culture**
- **Alternative sources**
  - **Retrospective banks of previously characterized selected positive samples**
    - **How characterized (guidelines for method acceptability)**
    - **Storage requirements**
  - **Retrospective banks of all previously characterized samples (positive and negative)**
    - **Process to eliminate bias for random**
  - **Ability to retest with previous method with discordant results vs new test**
    - **Degradation during storage**
    - **New device more sensitive than predicate device**

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Comparison to a "Gold Standard"

- **Compare to current non-molecular method**
- **Compare to another FDA IVD of high quality**
- **Problems**
  - No comparator available
  - Comparator method is less sensitive and/or specific than new assay
- **Effects assay sensitivity and specificity**
- **Discordant resolution**
  - Against well validated LDT with bi-directional sequencing
  - Testing needs to be done on all samples?
  - Discordant analysis should be included in primary performance outcomes

Laboratory Interpretation of Results

- **User friendly software**
- **Interpretation of complex algorithms**
- **Need to establish positive/negative thresholds per target or is one acceptable?**
  - May loose sensitivity and/or specificity
- **Need for an indeterminate zone?**
- **Is the level of detection relevant to any or all targets?**
  - Any presence significant
  - Differentiate colonization vs infection

Clinical Interpretation of Results

- **What is the clinical impact of a false negative/ false positive result?**
  - Treatment (wrong or lack of)
  - Infection control: cohorting, transmission
- **What is the clinical significance of mixed infections?**
  - Mixed viral, bacterial or both
  - May or may not yet know
- **How should we report mixed infections?**
- **Medical education**

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Clinical Validation

Results correlate with clinical disease
May not be necessary for established disease

- **Clinical sensitivity**
    - **Relative to clinical decision making**
    - **Relative to target, specimen source**
    - **Too sensitive may not always be best**
- **Clinical specificity**
    - **Ability of the test to give a positive result in the presence of disease (PPV)**
    - **Ability of the test to give a negative result in the absence of disease (NPV)**
    - **What is the clinical impact of a false positive result for a rare analyte?**

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Clinical Validation

- **Define reference range**
  - **Relative to target, specimen source**
  - **Relative to patient population**
  - **Relative to disease state**

- **Sources and references**
  - **Published literature**
  - **Clinical trials and evaluations**
  - **Chart reviews**

Post-analytical Validation

- **Software interpretations**
- **Calculations**
- **Instrument report formats**
- **Instrument maintenance**
- **Stability of nucleic acids during storage**
- **Stability of samples for retest**
- **Stability of reagents over time**

Laboratory Implementation

Laboratory Implementation Parameters

- **Assay and equipment verification**
- **Data analysis and reporting**
- **LIS/HIS**
- **SOPM**
- **Training and competency assessment**
- **Proficiency testing**
- **Clinical staff education**

Verification Studies

- **Analytical sensitivity/specificity**
- **Accuracy/precision**
- **Reproducibility**
- **Clinical sensitivity/specificity**
- **Reference range**
- **Instrumentation performance**
- **Quality control performance**

Verification Studies

- **Demonstrate that you have verified the analytical and post-analytical performance characteristics as established by the manufacturer**
  - Varies whether qualitative or quantitative assay:
- **Confirm reference values and reportable ranges**
- **Confirm clinical performance**
- **Adequate number and reasonable distribution of sample types tested**
- **Results compared to another valid assay**
- **Can cite references**

Clinical Verification

- **What to test, how to test, when to test and how much to test?????**

- **Need to balance establishing accurate performance characteristics with cost, time, and practicality**
  - Specimen availability: rare or common target
  - Primers, probes: previously published or new
  - Comparator assays: available or not
  - Experience with specimen type(s)
  - Experience with technology

Verification Materials

- **Studies performed in appropriate and all sample matrices to be tested clinically**
  - Sensitivity, specificity, inhibition
- **Clinical specimens of known reactivity or concentration (previously tested)**
- **Stock organisms (rare targets???)**
- **Commercial sources**
  - RNA, DNA, whole virus, panels
  - Manufacturer provided validation panels
- **Spiked samples**
- **Split samples – reference laboratory**
- **Proficiency test samples**

External Controls

- **Check for:**
  - Operator, instrument, reagents, sample, environment
- **Monitor all aspects of the analytical process:**
  - Sample addition, sample preparation, nucleic acid purity and quantity, reagent addition, reagent function, inhibition, reaction, detection and resulting
- **Type and frequency depend on:**
  - FDA status, CLIA, CAP. State requirements
  - Manufacturer requirements as stated in PI
  - Test format (single cartridge vs batch)
  - Every analyte: new lot, new shipment
  - Individual ($$$$) or pools
  - Rotate over test runs

Staff Training and Competency

- **Read and sign SOPM**
- **Training:**
  - Prior to clinical testing
- **Blinded competency panels**
  - In-house, commercially purchased
- **Competency**
  - PT samples, in-house blinded panels
  - Visual observation
  - Yearly
- **Documentation**

PT Testing (CLIA-88)

- **Minimum of 5 samples per testing event (based on method: culture, PCR, DFA etc)**
- **Minimum of three testing events at approximately equal intervals per year (CMS regulated analyte)**
- **Minimum of two testing events at approximately equal intervals per year (non-regulated analyte)**
- **Limited commercial PT source materials**
- **In-house proficiency test materials**
  - blinded commercial panels of known reactivity
  - samples split with a reference laboratory
  - previously tested samples of know reactivity

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Reimbursement

Reimbursement Issues

- **Will we be reimbursed and at what rate?**
- **Will reimbursement at a minimum cover testing costs?**
- **Varies by:**
  - **payor, plan within payor, HMO, capitated, State (CMS)**
- **FDA status does not guarantee payment**
- **Lack of target specific CPT codes**
- **Must use generic code xxxx times 1,2,3,4…..**
- **MUEs: limit number of same CPT per day/patient**
- **Will reimbursement change to "syndromic" regardless of number of pathogens detected?**

Thank you

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